

REFERENCE COUNT: 37

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Several observations indicate that the Raf-1 kinase is a downstream effector of **protein kinase C-epsilon** (PKC epsilon). We recently have shown that Raf-1 is constitutively activated in PKC epsilon transformed Rat6 fibroblasts, and transformation can be reverted by expression of a dominant negative Raf-1, but not a dominant negative Ras mutant (Cacace et al., 1996), Cai et al, (1997) demonstrated that PKC epsilon induced proliferation of NIH3T3 cells is independent of Ras or Src, but depends on Raf-1. These authors further suggested that PKC epsilon activates Raf-1 by direct phosphorylation. Here we have investigated the functional interaction between PKC epsilon and Raf-1, PKCE, but not PKC alpha, was found to **bind** to the Raf-1 kinase domain, The association appeared to be direct, as it could be reconstituted in **vitro** with **purified** proteins, Raf-1 and PKC epsilon could be co-precipitated from Sf-9 insect cells and PKC epsilon transformed NIH313 cells (NIH/epsilon). The association was negatively regulated by ATP in **vitro** and by TPA treatment in NIH/epsilon cells, but not in Sf-9 insect cells, Raf-1 was constitutively activated in NIH/epsilon cells, However, using coexpression experiments in Sf-9 cells and transiently transfected A293 cells we did not obtain any evidence for a direct activation of Raf-1 by PKC epsilon. PKCE did not induce translocation of Raf-1 to the membrane, Furthermore, PKCE did not activate Raf-1 nor enhance the kinase activity of Raf-1 that had been pre-activated by coexpression of Ras or the Lck tyrosine kinase, In contrast, conditioned media from PKC epsilon transformed cells induced a robust activation of Raf-1. This activation could be partially reproduced by recombinant TGF beta, a growth factors secreted by PKC epsilon transformed Rat6 cells. In conclusion, our results suggest that PKC epsilon stimulates Raf-1 indirectly by inducing the production of autocrine growth factors.

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FILE 'MEDLINE' ENTERED AT 09:53:50 ON 16 OCT 2002

L1 439 S PROTEIN KINASE C EPSILON
L2 634679 S BIND?
L3 1001261 S PUR?
L4 743343 S CELL CULTURE OR IN VITRO
L5 2 S L1 (S) L2 (S) L3
L6 1 S L1 (S) L2 (S) L3 (S) L4
L7 5 S L1 (S) L2 (S) L4

FILE 'PCTFULL, USPATFULL, BIOSIS, EMBASE, SCISEARCH, CONFSCI' ENTERED AT 10:00:38 ON 16 OCT 2002

L8 15 S L5
L9 11 DUP REM L8 (4 DUPLICATES REMOVED)
L10 10 S L6

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